ULTRASONIC–PROBE ASSISTED EXTRACTION OF PHENOLIC ACIDS IN IONIC LIQUID FROM *QUERCUS INFECTORIA* GALLS

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SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree in Master of Science

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Biji *Quercus Infectoria* (QI) atau dikenali sebagai Manjakani telah digunakan secara meluas dalam perubatan tradisional kerana khasiat yang luar biasa asid fenolik termasuklah asid galik (GA) and asid tanik (TA). Walau bagaimanapun, terdapat beberapa kekurangan dalam kaedah pengektrakan asid fenolik yang berkait dengan tahap ketoksikan pelarut yang tinggi dan kaedah yang kurang efisien. Dalam kajian ini, kesan kaedah pengekstrakan asid fenolik daripada biji QI dan keadaan optimumnya dianalisa. Asid fenolik daripada biji QI diekstrak melalui kaedah ultrasonik probe (UPAE) menggunakan cecair ionik sebagai pelarut, kaedah pengekstrakan akueus klasik (CAE), kaedah pengekstrakan hidro penyulingan (HDE) dan kaedah pengekstrakan ultrasonik klasik (CUBAE). Hasil pengekstrakan kesemua kaedah menujukkan kaedah UPAE dengan jumlah ekstrak GA tertinggi sebanyak 130.76 mg/g diikuti kaedah CUBAE sejumlah 81.56 mg/g, CAE sebanyak 43.76 mg/g dan HDE dengan jumlah ekstrak sebanyak 14.74 mg/g. Jumlah ekstrak TA juga menunjukkan kaedah UPAE sebagai kaedah yang paling efektif dengan penghasilan sebanyak 1556.26 mg/g, diikuti oleh kaedah CUBAE sebanyak 810.74 mg/g, kemudian kaedah CAE dengan jumlah ekstrak 179.97 mg/g dan akhirnya kaedah HDE sebanyak 118.02 mg/g. Kaedah UPAE merupakan kaedah yang paling efektif berbanding kaedah lain kerana kaedah ini menggunakan akustik kavitasi untuk memecahkan sel membran tumbuhan dan akhirnya mengurangkan limitasi dalam pemindahan jisim. Prestasi cecair ionik 1-Butyl-3-methylimidazolium tetrafluoroborate, [Bmim][BF_4] dan 1-Butyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide, [Bmim][Tf_2N] dibandingkan dengan air, pelarut organik dan surfaktan Cetyltrimethylammonium bromide (CTAB). Keputusan menunjukkan bahawa cecair ionik [Bmim][Tf_2N] memberikan hasil pengekstrakan tertinggi yang mungkin disebabkan oleh rantaian panjang alkil imidazolium dan anion Tf_2N yang kompleks, yang kemudiannya membentuk ikatan hidrogen yang kuat antara kumpulan hidroksil daripada asid fenolik, diekstrak keluar daripada biji QI. Ini seterusnya meningkatkan proses ekstrak. Kaedah UPAE dikendalikan dalam keadaan optimum pada suhu 70^{o}C dengan kitaran sonikasi 40%, dicairkan dalam larutan [Bmim][Tf_2N] pada kepekatan 0.10 M dengan nisbah 1:10 selama 8 jam. Proses ini menghasilkan jumlah ekstrak tertinggi sebanyak 870.90 mg/g GA dan 3157.97 mg/g TA. Aktiviti antioksidan asid fenolik yang tinggi pada IC_{50}, 26.57 μg/mL juga dicatatkan pada keadaan optimum yang sama. Sampel biji QI juga melalui kaedah FT-IR Spektrometri dan SEM bagi membuktikan keberkesanan intensiti ultrasonik yang tinggi meggunakan kaedah UPAE berjaya mengekstrak jumlah asid fenolik yang tinggi. Seterusnya, analisis menggunakan RSM bagi kaedah UPAE menunjukkan model yang baik dengan nilai kebarangkalian yang rendah (<0.0001) dan tinggi R^2. Keadaan optimum hasil daripada sistem RSM untuk proses pengekstrakan didapati pada 9.14 jam, nisbah 1:6 dan suhu 75^{o}C, menghasilkan jumlah fenolik asid maksimum sebanyak 4119.77 mg/g. Seterusnya, model matematik yang berbeza (Rate Law, Peleg dan Fick) dianalisis untuk proses pengekstrakan dan didapati bahawa model model Fick berjaya menerangkan proses UPAE pada ketepatan 97% yang mengesahkan bahawa kemeresapan adalah faktor penting dalam mengekstrak asid fenolik dari biji QI dengan bantuan cecair ionik. Secara keseluruhannya, proses pengekstrakan menggunakan kaedah UPAE dan cecair ionik sebagai pelarut boleh menjadi faktor ke arah pembangunan teknologi pengekstrakan pada masa hadapan.

ABSTRACT

Quercus Infectoria (QI) galls or commonly known as Manjakani was widely used in folklore medicine due to its remarkable active ingredients of phenolic acids including gallic acid (GA) and tannic acid (TA). However, the extraction methods to extract the phenolic acids have several limitations related to high toxicity and low efficiency. In this study, the effect of the phenolic acids from QI galls extraction method and operating conditions were studied. The phenolic acids from QI galls were extracted via ultrasonic–probe assisted extraction (UPAE) method using ionic liquid solvent, conventional aqueous extraction (CAE) method, hydro–distillation extraction (HDE) method and classical ultrasonic–bath assisted extraction (CUBAE) method. The result indicate that the UPAE method yielded highest extraction amount of GA followed by CUBAE, CAE and HDE method at 130.76 mg/g, 81.56 mg/g, 43.76 mg/g and 14.74 mg/g, respectively. On the other hand, the extraction yield of TA was higher than the GA, having a same pattern with the highest value of extraction yield via UPAE, followed by the CUBAE, CAE and HDE method with 1556.26 mg/g, 810.74 mg/g, 179.97 mg/g and 118.02 mg/g, respectively. The UPAE method extraction yield exceededs the other conventional methods because it utilized acoustic cavitation to disrupt the plant tissues, broke down cell membrane and eventually decreased mass transfer limitations. The performance of the ionic liquids, namely 1-Butyl-3-methylimidazolium tetrafluoroborate, [Bmim][BF_4] and 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, [Bmim][Tf_2N] as solvents were also compared with water, organic solvent and Cetyltrimethylammonium bromide (CTAB) surfactant. The results show that [Bmim][Tf_2N] ionic liquid gave highest extraction yield which might due to the long alkyl chain of imidazolium cation and the complexity of the Tf_2N anion, that might form strong hydrogen bond with the hydroxyl group of phenolic acids, extracted it out from the solid sample and eventually increased the efficiency of the extraction process. The operating conditions for the UPAE process were also investigated. It is found that the highest amount of GA (870.90 mg/g) and the TA (3157.97 mg/g) were achieved at 70^{o}C with 40% duty cycle, sample dilution in solvent [Bmim][Tf_2N] at concentration of 0.10 M with sample–to–solvent mass ratio of 1:10 for 8 hours. A high antioxidant activity of IC_{50}, 26.57 µg/mL was obtained at the same condition. The characterization results of QI galls using FT–IR Spectrometry and SEM confirmed that high ultrasonic intensity from UPAE method was able to extract high amount of phenolic acids into the extraction media. Moreover, the RSM analysis for the UPAE method of the phenolic acids showed a good significance of model with low probability values (<0.0001) and a high coefficient of determination (R^2). The optimum conditions for the extraction process were found to be at 9.14 hours, sample–to–solvent mass ratio of 1:6 and temperature at 75^{o}C, attaining maximum phenolic acids of 4119.77 mg/g. By modeling via rate law, Peleg's and Fick's mathematical models, Fick's model was successfully predicted the UPAE process with 97% accuracy compared to other kinetic models i.e. rate law model and Peleg's model. This also confirmed that diffusivity factor controlled the extraction of phenolic acids from the QI galls using the ionic liquid. Significantly, the UPAE extraction process using ionic liquid as solvent could be a great advantage in the future development of extraction technology.

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LIST OF SYMBOLS

I	Intensity
P	Power
A	Area
$A_{control}$	Absorbance of DPPH without sample
A_{sample}	Absorbance of DPPH with sample
dc/dt	Rate of extraction
k_1	Second order rate constant
c	Concentration of the liquid at time, t
c_∞	Concentration of compound in the liquid
h	Initial extraction rate
K_1	Peleg's rate constant
K_2	Peleg's capacity constant
C_t	Concentration at time, t
C_0	Initial concentration
f_1	Fraction at fast (washing) stage
f_2	Fraction at slow (diffusion) stage
r	Particle radius
D_1	Diffusion coefficient at fast (washing) stage
D_2	Diffusion coefficient slow (diffusion) stage
exp	Exponential function
π	Pi (3.142)

LIST OF ABBREVIATIONS

QI	Quercus Infectoria
GA	Gallic acid
TA	Tannic acid
CAE	Conventional aqueous extraction
HDE	Hydro–distillation extraction
CUBAE	Classical ultrasonic–bath assisted extraction
UPAE	Ultrasonic–probe assisted extraction
UAE	Ultrasonic assisted extraction
IL	Ionic liquid
[Bmim][Tf_2N]	1–Butyl–3–methylimidazolium bis(trifluoromethylsulfonyl)imide
[Bmim][BF_4]	1–Butyl–3–methylimidazolium tetrafluoroborate
$AlCl_3$	Aluminium trichloride
CTAB	Cetyltrimetylammonium bromide
TPC	Total phenolic content
TFC	Total flavonoid content
UV–Vis	Ultraviolet–visible spectroscopy
FT-IR	Fourier Transform Infrared
SEM	Scanning Electron Microscopy
RSM	Response surface methodology
FFD	Full factorial design
FCCCD	Face–centred central composite design
OFAT	One factor at a time
HPLC	High Performance Liquid Chromatography
DPPH	2, 2–Diphenyl–1–picryl–hidrazil
GAE	Gallic acid equivalent
QE	Quercetin equivalent
BE	Before extraction
AE–UPAE	After extraction–Ultrasonic probe assisted extraction
RMSD	Root-mean-square deviation

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